Hydroperoxide in Internal Jugular Venous Blood Reflects Occurrence of Subarachnoid Hemorrhage-Induced Delayed Cerebral Vasospasm

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Background: To investigate the association between subarachnoid hemorrhage-induced delayed cerebral vasospasm (DCVS) and oxidative stress, an oxidation product, hydroperoxide, was measured in 3 specimens: peripheral arterial blood, cerebrospinal fluid (CSF), and internal jugular venous blood (IJVB). *Methods:* Hydroperoxide was measured using the diacron reactive oxygen metabolites (d-ROMs) test. The hydroperoxide levels were evaluated based on the rate of change in the d-ROMs test value on day 6 relative with that on day 3 (d-ROMs change rate). *Results:* The subjects were 20 patients. The d-ROMs change rate in IJVB was significantly higher in patients with DCVS on day 6 than in those without it ($P < .01$). When the patients were classified into the following 3 groups: Group A (no DCVS occurred throughout the clinical course); Group B (DCVS occurred, but no cerebral infarction [CI] was induced); and Group C (DCVS occurred and caused CI), the d-ROMs change rate in IJVB was the highest in Group C, followed by Group B then A ($P < .01$). The d-ROMs change rates in peripheral arterial blood and CSF were not related to the development of DCVS. *Conclusions:* It was concluded that the more severe DCVS occurs and is more likely to progress to CI as the IJVB hydroperoxide level rises early after the development of subarachnoid hemorrhage. **Key Words:** Hydroperoxide—delayed cerebral vasospasm—oxidative stress—internal jugular venous blood—subarachnoid hemorrhage.

The cause of subarachnoid hemorrhage (SAH) is rupture of cerebral aneurysm in most cases.[1] Rerupture of cerebral aneurysm in the acute phase markedly influences the prognosis, for which surgical treatments with aneurysmal neck clipping and coil embolization have achieved favorable outcomes.[2] Another serious complication influencing the prognosis of SAH patients is cerebral vasospasm. This is classified into early cerebral vasospasm, which occurs early (within 24 hours) after the development and delayed cerebral vasospasm (DCVS), which occurs 4-14 days after the development.[3] DCVS occurs in about 70% of SAH patients, and 20%-30% are symptomatic. Severe neurologic deficits remain and maybe life threatening.[4] Many points have not been clarified with regard to the pathology, and no therapeutic method completely inhibiting it has been established.

DCVS is considered to be induced by hemoglobin in hematomas present around the cerebral arteries in the subarachnoid space,[5,6] and it has been clarified that red

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blood cell-derived reactive oxygen species cause peroxidation and induce DCVS.[7,8] Recent studies clarified that free radicals released from oxyhemoglobin contained in red blood cells induce lipid peroxidation of the cell membranes of vascular smooth muscles, resulting in activation of calcium channel, protein kinase C, and Rho-kinase, which maybe a mechanism of DCVS.[9-15] However, in vivo evaluation of oxidative stress is difficult because of instability of free radicals, and only a few studies have quantified it.[16-19] Thus, we quantitatively evaluated oxidative stress by measuring an oxidation product, hydroperoxide, in 3 specimens of SAH patients, which are peripheral arterial blood, cerebrospinal fluid (CSF), and internal jugular venous blood (IJVB), using a free radical analytical system (Wismerll Co, Ltd, Tokyo, Japan) capable of measuring it in a simple manner. In addition, the association between the hydroperoxide levels and development of DCVS and progression to cerebral infarction (CI) was investigated.

Materials and Methods

The subjects were consecutive SAH patients (World Federation of Neurosurgical Societies [WFNS] grade III-V) who underwent surgical treatment for the acute phase at Toho University Omori Medical Center and Misato Central General Hospital between April 2011 and March 2013. At our institution, a radial arterial catheter is inserted to monitor direct blood pressure (BP), a lumbar CSF drain is applied to irrigate subarachnoid blood, and a jugular bulb venous oxygen saturation monitoring (SjO_2) catheter is inserted into the internal jugular vein to measure cerebral hemodynamics in patients with WFNS grade III or more severe SAH as a routine practice. Through these catheters, peripheral arterial blood, CSF, and IJVB were collected, respectively, and an oxidation product, hydroperoxide, was quantitatively measured to evaluate the in vivo oxidative stress level. Head computed tomography (CT) and cerebral angiography were performed in all patients as preoperative neuroradiologic examinations, and surgical aneurysmal neck clipping or coil embolization was performed within 48 hours after the onset. The catheter for direct BP monitoring, lumbar CSF drain, and SjO_2 catheter were inserted during or immediately after surgery, and peripheral arterial blood, CSF, and IJVB were collected through the catheters, respectively. Regarding the choice of which of the bilateral jugular bulbs to use to insert the SjO2 catheter, the side with greater venous blood flow to the jugular bulb on preoperative cerebral angiography was selected. When their blood flow was similar, the right side was selected.[20,21] The samples were collected between 8:00 and 9:00 a.m. in consideration of diurnal fluctuations of the oxidative stress level. Because intravenous anesthetics, such as propofol, have been reported to increase the antioxidant power,[22] patients not treated with an intravenous anesthetic after surgery were selected. To evaluate oxidative stress, the samples were subjected to the diacron reactive oxygen metabolites (d-ROMs) test using free radical analytical system, and the hydroperoxide levels were spectrophotometrically measured. The hydroperoxides are intermediate oxidative products of lipids, peptides, and amino acids, and their levels constitute an index of oxidative injury of cellular components. The hydroperoxide levels were measured as follows[23,24]: 20 μL of blood and CSF was dissolved in an acetate-buffered solution (pH 4.8). The hydroperoxide groups react with the transition metal ions liberated from the proteins in the acidic medium and are converted to alkoxy and peroxy radicals, according to the Fenton reaction. These newly formed radicals, the quantities of which are directly proportional to those of the peroxides present in serum and CSF, are trapped chemically with chromogen (*N*,*N*-dimethyl para-phenylenediamine), leading to formation of the corresponding radical cation. The concentration of this persistent species can be determined at 505 nm using a spectrophotometer. Results are expressed in Carratelli (Carr) units, where 1 Carr unit corresponds to .8 mg/L of hydrogen peroxide. The measured values are presented as the d-ROMs test values in the following.

Because d-ROMs test values markedly vary among individuals, the absolute values cannot be directly compared. Thus, the rate of change in this value over time in each patient was investigated.[25,26] Moreover, surgery increases oxidative stress,[27,28] and this influence has to be eliminated. Because brain injury-induced oxidative stress is reduced within 48 hours,[29] regarding the d-ROMs test value on day 3 as the control, oxidative stress was evaluated based on the rate of change in the d-ROMs test value on day 6 (d-ROMs change rate).

The following 2 items were investigated based on the values measured in peripheral arterial blood, CSF, and IJVB: First, the presence or absence of DCVS on day 6 was investigated, and the d-ROMs change rates (day 6/day 3 d-ROMs test value) at this time point were compared. Second, the patients were divided into 3 groups: (1) patients in whom no DCVS occurred throughout the course (Group A); (2) DCVS occurred but did not induce CI (Group B); and (3) DCVS occurred and caused CI (Group C), and these 3 groups and the d-ROMs change rates on day 6 were compared. Oxidative stress increases in CI,[30-33] which may produce an error in analysis of the relationship between the development of DCVS and oxidative stress level. To avoid this, the patients who developed CI were excluded from the later investigation of the d-ROMs test value. Because the d-ROMs change rate on day 3 was regarded as the baseline, patients who had already developed cerebral vasospasm on day 3 were also excluded.

This clinical study was performed after approval by the ethics committees of Toho University School of Medicine and Misato Central General Hospital. The catheters and